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CUTANEOUS INNERVATION

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This is a review of historical facets of research in cutaneous neurohistology. The silver stains, used for some 150 years, led to the discovery of the neurone theory and to contemporary comparative studies. The cholinesterase methods, used since 1950, are the most convenient for general studies of nerves. The fluorescence technique of Falck (1962) is valuable for the study of adrenergic fibers. The electron microscope techniques used since 1950 have allowed the comprehensive description of myelinated and unmyelinated nerve fibers and of corpuscles. The most important unsettled questions pertain to the physiology of cutaneous nerves and precise definitions of cutaneous sensibility.

The advances in electron microscopy during the last 25 years have given us a deeper understanding of cutaneous innervation than had been gained during the previous 125 years. Nevertheless, it may be instructive to go back to the early era of cutaneous neurohistology and, from that vantage point, review recent investigations. Therefore, the aim of this report is to summarize the main areas of research in this field and to comment briefly about their significance; to discuss current techniques and the general structure of cutaneous nerves; to emphasize some important details and problems; and to make some suggestions for the future.

Only a few selected references have been given and, as far as possible, personal data are cited. The interested reader can find more material elsewhere [1].

TECHNIQUES AND STRUCTURES

Silver Stains

The general architecture of the skin nerves, and especially that of the receptors, has been studied since the second half of the 19th century. Krause used silver methods to focus attention on the encapsulated end-organ, later named after him [2], and he produced an accurate description of the end-bulb.

By the turn of the century, Ramon y Cajal and other prestigious neurohistologists had developed techniques and a deeper knowledge of the central nervous system. Out of their work came the so-called "neurone theory." These histologists were not much interested in skin, but their work in general became the keystone of modern neurohistology.

From before World War I until after World War II, both interest in and work on cutaneous innervation grew. In their eagerness to visualize all the nerve fibers, cutaneous biologists resorted to refined silver methods. They revealed some of the largest sensory fibers and their end-organs, as well as some of the small unmyelinated fibers only a few tenths of a micron in diameter. During this period, Bielschowsky [3], Stöhr [4], Boecke [5], and Jabonero [6] produced large amounts of data and greatly stimulated research, although their theories about the structure of autonomic nerves were never accepted in France, England, or the United States.

Stöhr [4] and Jabonero [6] were responsible for the two theories that prevailed in the late 1950s, the reticulum theory and the autonomic end-function theory, respectively. According to Stöhr, large postganglionic fibers join very tiny fibers embedded in a poorly defined syncytium. The tiny fibers anastomose freely and constitute a dense network in the vicinity of effector organs; within the network, they have neither starting nor ending points. The autonomic end-function theory proposed by Jabonero states that a syncytial network, which is tightly bound to glands and vessels, is made of cytoplasmic ribbons with poor vacuolar, granular, and fibrillar differentiation. It represents a primitive nerve complex that, during the course of evolution, came under the control of the postganglionic fibers.

We now regard these theories skeptically. The overreliance on heavy silver staining seems to have been an error, and the mechanisms have never been completely understood [1, p 129]. We must not, however, fail to recognize the usefulness of the silver techniques for analyzing sensory fibers and endings and obtaining excellent results. I myself use, with or without counterstains, the ammoniated silver carbonate method of Bielshowsky–Gros–Jabonero [7] for frozen sections, the ammoniated silver carbonate method of Van Campenhout [8] for paraffin sections, and the simple silver method of Winkelmann and Schmit [9] for nerve axoplasm.

After World War II research flourished. Various techniques were applied to human and animal skin

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and much information was gained. Too much attention was paid to individual and species variations and, unfortunately, too little to similarities.

Cholinesterase Techniques

The first cholinesterase techniques devised some 25 years ago were more complicated than the currently used Gomori's procedure [10] which is quite easy, reliable, and well understood. Technical comments and a survey of inhibitors have been published [1, p 91]. Attention was first focused on sensory bulbs whose cells stain easily with the specific acetylthiocholine substrate and more heavily with the nonspecific butyrylthiocholine substrate. Neurites and nuclei do not stain. In man, the Wagner–Meissner corpuscles and the Krause end-bulbs in the superficial dermis and the Vater-Pacini corpuscles in the low dermis and hypodermis stain; in most other mammals, the mammalian end-organs including the non-encapsulated hair follicle end-organ stain.

The superficial or subepidermal network is made up of tiny fibers that are acetylcholinesterase positive and loosely interwoven in the papillary and subpapillary layers. This sensory apparatus of the skin becomes denser in specialized areas and is superimposed with end-bulbs [1, p 80]. The sympathetic or vegetative network is acetylcholinesterase positive, of impressive density, and closely applied to the vessels, smooth muscles, and sweat glands (eccrine glands being more richly innervated than apocrine glands).

End-bulbs in some species are not only butyrylcholinesterase positive but filled with unrelated and nonphysiologic hydrolases such as indoxylesterase (e.g., the badger [11]). In other species the end-bulbs contain alkaline phosphatase (e.g., the kangaroo [12]). These findings introduce a new chapter in comparative neurohistology dealing with the distribution and type of enzymes in the various species.

Because of diffusion, the cholinesterase techniques are plagued with a lack of precision; definition is more histologic than cytologic; and poor staining may be related to various chemicals (e.g., previous administration of toxins or poisons with anticholinergic properties; use of local anesthetics containing adrenalin [1, p 97]). In spite of this, they are advantageous. They provide a complete view in horizontal and vertical sections of the sensory apparatus (with the exception of the Merkel cells) and of the sympathetic network. They make possible a quantitative approach [13], and whole-mount techniques are possible for thick sections or sheaths of tissues [14]. Because the techniques are simple, easy, and reliable, artifacts are unlikely to occur. The sections can be counterstained by Giemsa and by silver after indoxylesterase [11].

The Fluorescence Method of Falck

The fluorescence method developed by Falck and his collaborators in the early 1960s is characterized by unchallenged specificity and by important theoretical implications. Unfortunately, it requires a large investment of time and money [15, 16]. Tissue is freeze-dried, incubated with paraformaldehyde, and observed under ultraviolet light. The adrenergic fibers of smooth muscles and arteries are revealed.

Intraepidermal Fibers

Intraepidermal fibers are one of the most fascinating, frustrating, and controversial problems for cutaneous investigators. These problems arise from inadequacies of techniques and from variability of species studied. My own position is as follows. In animals, especially in the lips and the nose, there are numerous intraepidermal nerves or transepidermal nerves if one accepts that they are subjected to rapid turnover as are the epidermal cells. Silver, cholinesterase, and ultrastructural methods can be used to visualize them fairly well in many species (e.g., the common mole, the opossum, the hedgehog).

Serri and his collaborators have seen fibers expanding into the epidermis before prenatal maturation of the superficial plexus and corpuscles [17]. In healthy neonates, extensive investigation of the prepuce did not show any intraepidermal fiber [18]. After extensive study with the light and electron microscope [1, p 71], no fibers have been detected in healthy or diseased adults except for intraepidermal fibers in the lip of a man afflicted with Urbach–Wiethe disease [1, p 135]. At present, I believe that intraepidermal fibers in man are rarely, if ever, present.

Ultrastructure

Electron microscopically, all the encapsulated end-organs share features, although spatial relationship or gross architecture may differ. In the Wagner–Meissner corpuscle, for example, flattened sheaths of cytoplasm are piled up around the axon ending and are sustained by loose connective tissue. The cytoplasm is distributed into thin layers characterized by much pinocytic activity and covered by a basement membrane; in between, lie ill-defined interstitial material and reticulin fibers. Also present are poorly outlined membrane junctions somewhat similar to tight junctions and to specialized contacts between the Schwann cells of peripheral nerves. Are they anchoring devices or the site of some electrochemical phenomenon?

The axon endings—very large, poor in filaments, and filled with mitochondria—are closely allied to the corpuscular cells. The abundance of mitochondria in these endings is quite characteristic but not understood. Other corpuscles are similar except for the concentric wrapping of cytoplasmic sheaths in the Pacini end-bulb and for poor organization in the mammalian end-organ. In the hair follicle end-organ, large, lancet-shaped axon endings are loaded with mitochondria in close contact to the hair, and are tightly bound to their schwannian envelope.

Because of the general appearance of the corpuscular cells, because they appear to be continuous with the Schwann cells of the sensory fibers, and because of their histochemical properties, I regard corpuscular cells as differentiated Schwann cells.

The general structure of the myelinated fibers was first established by Fernández-Morán in the early 1950s [19] and studied in detail by other electron microscopists, especially Elfin [20]. Structurally, the cutaneous fibers do not differ significantly from the nerve trunks. Remember that the number of "lines" (i.e., the thickness) of myelin is somewhat related to the axon diameter and is rather lower than in the trunk fibers.

The rare attachment plates [1, p 40] look like cytoplasmic densifications of the schwannian cytoplasm adjacent to the membranes where myelin unrolls at the Ranvier node. They might be correlated with saltatory conduction or with stabilization of myelin. Attachment plates, tight junctions, formation of myelin, and the ability to shelter axons and collagen bundles are specific properties of the Schwann cells and should be further investigated.

Fernández-Morán published the first description of the unmyelinated fiber in 1952 [21] and later on other investigators confirmed his description and added further details [1, p 21]. It has been found that (1) unmyelinated axons outnumber myelinated ones; (2) every unmyelinated fiber contains several axons; (3) axons are more or less deeply engulfed in folds of the schwannian cytoplasm, the so-called mesaxon; (4) Schwann cells and axons are isolated from the interstitial tissue by a common basement membrane; (5) axons have a diameter of a few tenths of a micron.

In 1968, I stressed the fact that the Schwann cells, the axons, and the basement membrane are bound together into an indissoluble structure for which I coined the name "fundamental architectural unit" [1, p 76]. This holds true also for the myelinated fibers and for the end-organs, the corpuscular cells being similar to Schwann cells.

Are there exceptions? I prefer to say there are apparent exceptions. Some unmyelinated axons may be partially deprived of their schwannian envelope for the very short distance that they are applied to effector cells. Intraepidermal fibers are isolated from the connective tissue by the basement membrane of the nerve which is reflected into the basement membrane of the epidermis. Furthermore, these fibers are embedded in the epithelial plasmalem (to some extent epidermal cells play the role of the Schwann cells). I am uncertain about naked axons, more precisely about the naked endings of sensory fibers in the papillary layer. While waiting for better evidence, I believe that the law of the "fundamental architectural unit" remains unchallenged.

A few other structures should be mentioned. The perineural sheath of several layers, each of which has its own basement membrane, is lacking for the smallest nerves and isolated fibers. Its mechanical role and functions have been studied on larger trunks. The endoneural space contains Schwann cells and a few cells devoid of basement membrane thought to be fibroblasts. Long-spacing collagen fibrils may be seen in normal structures as well as in pathologic ones and the descriptive term, striated bodies, has been proposed for them [1, p 50]. Collagen pockets consist of bundles of collagen fibrils deeply engulfed in folds of the Schwann cell plasmalem but remaining outside the basement membrane. They may express the ability of the Schwann cells to wrap themselves around elongated structures [1, p 46].

CONSIDERATIONS FOR THE FUTURE

To arrive at a complete knowledge of neural tissues, I believe we must resort to the simultaneous use of several techniques: routine, silver, enzyme, and electron microscopic investigations. These techniques are not exclusive, but additive. Enzyme techniques should become more and more specific and precise and should be adapted to ultrastructural microscopy.

Many pathologic processes including polyneuritis, congenital disturbances of the nerve pathways, errors of metabolism which affect the nerves, diabetes, Fabry's disease, nerve tumors, and persistent itching must be studied. More extensive data about development and aging are needed to improve our understanding of structure and function.

Neurophysiology appears to be one of the most promising and exciting fields of research for the next few years. We already have a rather satisfactory knowledge of the morphology of the sensory devices; now we have to learn how they work. The relative roles of the corpuscular cells with their pinocytic activity and their hydrolase content and of the axons with their load of mitochondria have to be clarified. We do not know enough about trigger mechanisms, depolarization, or action potential. Morphologists and physiologists must collaborate to explain the whole process.

Some parameters of sensation warrant further investigation: the intensity and duration of stimulus, and the role of skin temperature, of hydration of the horny layer, of the influence of drugs, and of electrical or magnetic fields.

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